

Research Article

Pretreatment with the Combination of Drugs Protects against Cognitive Impairment in Diabetic Rats

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Abstract

Objectives: The World Health Organization has foreseen that 35.6 million people suffer from dementia is due diabetes and this number might be doubled by 2030 and unlike other complications due to diabetes, cognitive impairment occur early in patients. Pharmacological treatments cannot cure or stop the progression of cognitive impairment due to diabetes. Hence, in current study the attempt has made to prevent or delay the cognitive impairment in diabetic rats using combination of drugs.

Methodology: Diabetes was induced in male Wistar rats by administrating streptozotocin (52mg/kg,i.p). Diabetic rats were treated with combination of gamma linolenic acid (30mg/kg,p.o), alpha lipoic acid (30mg/kg,p.o), phloroglucinol (250mg/kg,p.o), benfotiamine (100mg/kg,p.o), in one group and gamma linolenic acid (30mg/kg,p.o), alpha lipoic acid (30mg/kg,p.o), allantoin (200mg/kg,p.o), benfotiamine (100mg/kg,p.o), in other group for eight weeks. The degree of protection was determined by measuring motor co-ordination, Barne maze, Glycosylated Haemoglobin (GHb) and transmitter like dopamine, nor-adrenaline, serotonin, gamma amino butyric acid and acetyl cholinesterase.

Results: Administration of combination of drugs significantly reduced glycated haemoglobin,dopamine,serotonin, latency to enter escape cage and significantly elevated the body weight, motor co-ordination, nor-adreline, gamma amino butyric acid, acetylcholinesterase levels compared to diabetic control rats.

Conclusion: Supplementation of combination treatment showed improved glycemic and neurotransmitters control in diabetic rats. Hence, the pretreatment with combination of drugs may be a way to delay cognitive impairment resulting from hyperglycemia and distortion of neurotransmitters.

Keywords: Cognitive impairment; Dementia; Streptozotocin; Gamma linolenic acid; Alpha lipoic acid; Benfotiamine; Phloroglucinol; Allantoin

Introduction

It has reported that diabetes mellitus can cause serious neuronal impairment and cognitive deficits [1,2]. The effect of diabetes mellitus on cognitive functions have been found to occur early in patients unlike other complications due to diabetes which usually requires years of diabetes before becoming clinical apparent. The most common cognitive deficits identified in patients with type 1 diabetes are slowing of information processing speed [3-6] and worsening psychomotor efficiency [3,4,7]. However, other deficits like attention, memory, motor strength and executive function have also been reported. Although much research has been done, the pathophysiology of cognitive dysfunction in diabetes is not well understood but it is likely that hyperglycemia, vascular disease, hypoglycaemia and insulin resistance play significant roles. The World Health Organization has foreseen that 35.6 million people suffer from dementia and this number might be doubled by 2030. One study suggested that over 80% of cases of Alzheimer Disease (AD) had either type 2 diabetes or impaired fasting glucose [8]. Furthermore, diabetes patients have a 50% - 75% increased risk of developing AD compared to non-diabetes patients [9]. Pharmacological treatments cannot cure or stop

the progression of cognitive impairment due to diabetes. Hence, in current study the attempt has made to prevent or delay the cognitive impairment in diabetic rats using combination of drugs. Addressing these deficits by single drug has yet to produce effective treatment for diabetic induced cognitive impairments. The current study addresses the treatment for diabetic induced cognitive impairment by using a combination of drugs targeted at various mechanisms underlying the progression of diabetic induced cognitive impairments.

Materials and Methods

Animals

Experimental animals, adult male Wistar rats (n=5) weighing between 200-250 g were included for the study. All rats were maintained under standard housing condition at controlled temperature at 25°C ± 2°C with 12 hr light/dark cycle with food and water provided standard rat diet and water ad libitum. Animals which did not comply with above criteria, and which were found to be diseased were excluded from the study. After one-week adaptation period, the healthy animals were used for the study. All the protocols were approved by Institutional Animal Ethical Committee. IAEC NO: AACP/P-48, India.

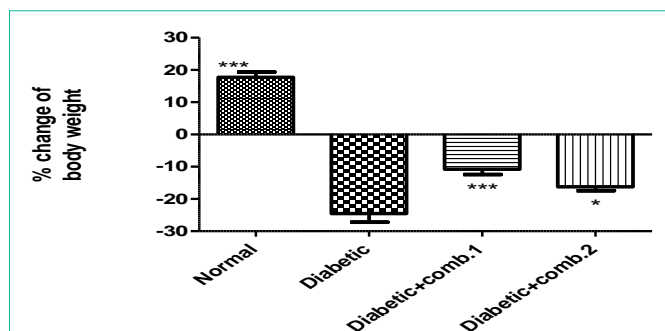


Figure 1: Effect of treatment of combination of drugs for eight weeks on % body weight change in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001, *P<0.05 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

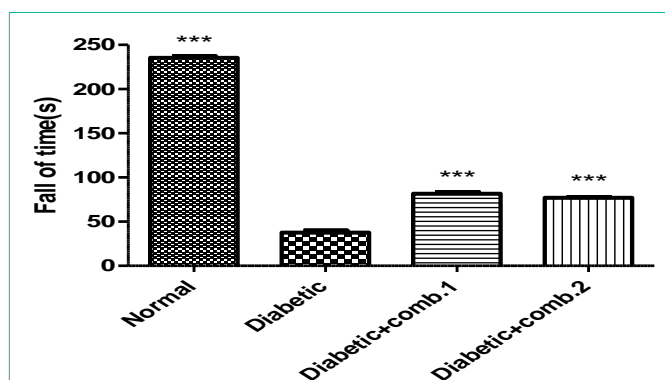


Figure 2: Effect of treatment of combination of drugs for eight weeks on muscle incoordination by rota rod performance in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

Experimental design

Thirty two rats were randomly divided into four groups. Group I served as normal control group. Group II, Group III and IV were induced diabetic rats and included in the study as experimental rats. Group II served as diabetic control group whereas Group III received gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine and Group IV received gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine daily for eight weeks. Treatment was started after diabetes was confirmed in rats. Rats were also administered insulin (3IU/day, s.c.) [10] for the complete period of the study. After 8 weeks initial and terminal body weight, behavioural and biochemical parameters were determined to evaluate the severity of diabetes induced cognitive impairment in treated group as compare to normal and diabetic control group.

Assessment of body weight

To assess the general condition of animals, they were examined daily for clinical signs such as alopecia, piloerection or hind limb weakness and mortality. Body weight was measured using digital balance (Essae® DS-252). Loss of body weight was compared between body weight measured at the beginning and at the end of the study.

Behavioural parameters

Motor co-ordination test and Barnes maze test were done according to published papers [11,12].

Biochemical parameters

Measurement of Glycosylated Hemoglobin (GHb) [13], brain neurotransmitters like dopamine, noradrenaline, serotonin. Gamma amino butyric acid, and acetyl cholinesterase were done according to published papers [14,15,16,17,18,19].

Statistical analysis

Statistical evaluations were done by ANOVA, expressed as mean \pm S.E.M. followed by Tukey's multiple comparison test using Graph Pad Prism 5 computer program. P<0.05 was considered statistically significant.

Results

Assessment of body weight

The percentage change in body weight of normal and diabetic rats at 8th week was found to be 17.72 ± 1.66 g and -24.53 ± 2.706 g. The body weight of diabetic rats was significantly reduced (P<0.001) as compared to normal control, similarly the change of body weight of diabetic treated with comb.1 and comb.2 was found to be -12.49 ± 0.93 g and -12.30 ± 2.457 g which significantly improved as compared to diabetic control rats (Figure 1).

Behavioural studies

Measurement of motor coordination using rota rod: Fall of time at 15 rpm of normal and diabetic rats was found to be 235.3 ± 1.753 and 37.58 ± 2.343 secs respectively, and the latency of diabetic rats was significantly reduced (P<0.001) as compared to normal control. Latency in diabetic rats treated with comb.1 and comb.2 was found to be 81.56 ± 1.90 secs and 76.93 ± 0.865 secs and same was significantly P<0.001 improved when compared to diabetic control rats (Figure 2).

Measurement of spatial memory and learning using barnes maze: Time taken to enter escape cage by normal and diabetes rats was found to be 15.59 ± 0.51 and 52.20 ± 1.58 secs respectively, and the

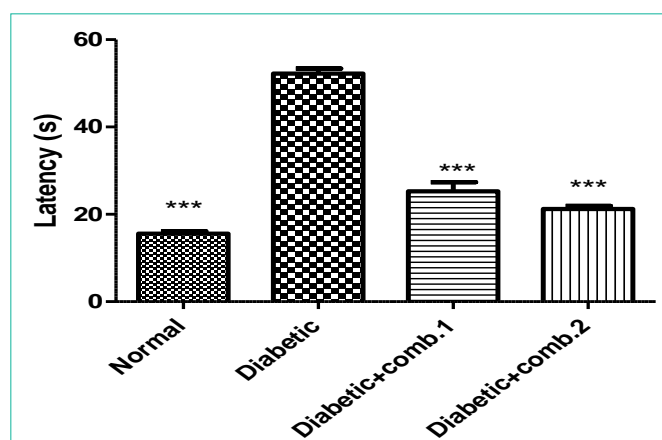


Figure 3: Effect of treatment of combination of drugs for eight weeks on spatial learning and memory by Barnes maze in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

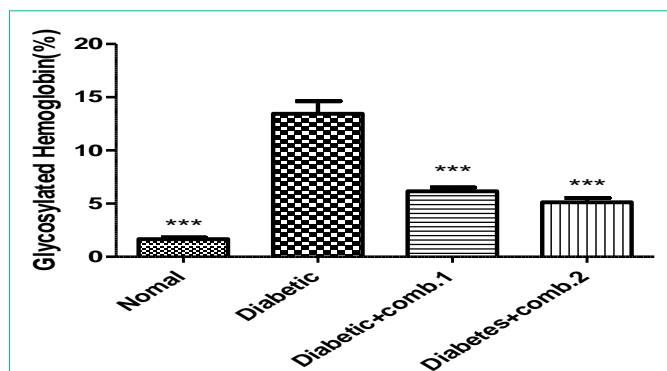


Figure 4: Effect of treatment of combination of drugs for eight weeks on %GHB in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

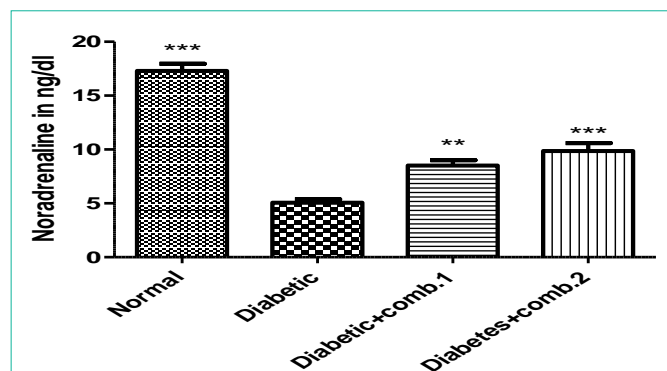


Figure 6: Effect of treatment of combination of drugs for eight weeks on brain nor-adrenaline level in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001, **P<0.01 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

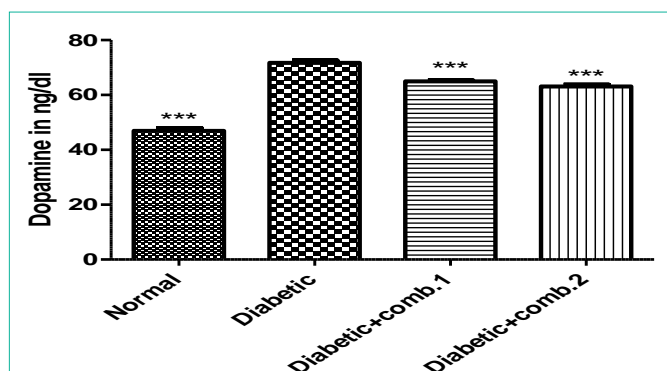


Figure 5: Effect of treatment of combination of drugs for eight weeks on brain dopamine level in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

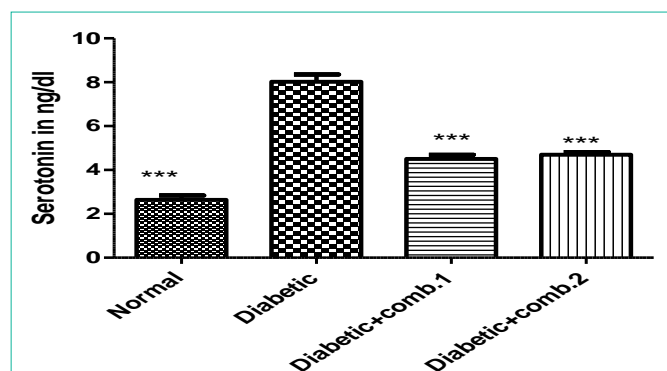


Figure 7: Effect of treatment of combination of drugs for eight weeks on brain serotonin level in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

latency of diabetes

rats was significantly increased (P<0.001) as compared to normal control. Latency in diabetic rats treated with comb.1 and comb.2 was found to be 25.29 \pm 2.066secs and 21.22 \pm 0.70secs and same was significantly P<0.001 improved when compared to diabetic control rats (Figure 3).

Biochemical studies

Estimation of GHb: The percentage GHb of normal and diabetes rats was found to be 1.667 \pm 0.1476 and 13.43 \pm 1.198 and same was significantly increased (P<0.001) as compared to normal control. The percentage GHb of diabetic rats treated with comb.1 and comb.2 was found to be 6.150 \pm 0.3510 and 5.117 \pm 0.407 and same were significantly improved. P<0.001 when compared with diabetic control rats (Figure 4).

Estimation of dopamine: The dopamine content in brain of normal and diabetic rats was found to be 46.9 \pm 1.005ng/dl and 71.7 \pm 0.88ng/dl respectively and the dopamine level of diabetic rats was significantly increased (P<0.001) as compared to normal control rats. Dopamine level in diabetic rats treated with comb.1 and comb.2

was found to be 64.94 \pm 0.33ng/dl and 63.10 \pm 0.64ng/dl and same was significantly increased (P<0.001) when compared to diabetes control rats (Figure 5).

Estimation of nor-adrenaline: The nor-adrenaline level in brain of normal and diabetic rats was found to be 17.30 \pm 0.66ng/dl and 5.060 \pm 0.31ng/dl respectively and the nor-adrenaline level of diabetic rats was significantly reduced (P<0.001) as compared to normal control rats. Nor-adrenaline level in diabetic rats treated with comb.1 and comb.2 was found to be 12.14 \pm 0.66ng/dl and 9.8 \pm 0.74ng/dl and same was significantly increased when compared to diabetes control rats (Figure 6).

Estimation of serotonin: The serotonin content in brain of normal and diabetic rats was found to be 2.64 \pm 0.19ng/dl and 8.02 \pm 0.33ng/dl respectively and the serotonin level of diabetic rats was significantly increased (P<0.001) as compared to normal control rats. Serotonin level in diabetic rats treated with comb.1 and comb.2 was found to be 4.5 \pm 0.2ng/dl and 6.310 \pm 0.64ng/dl and same was significantly reduced (P<0.001) when compared to diabetes control rats (Figure 7).

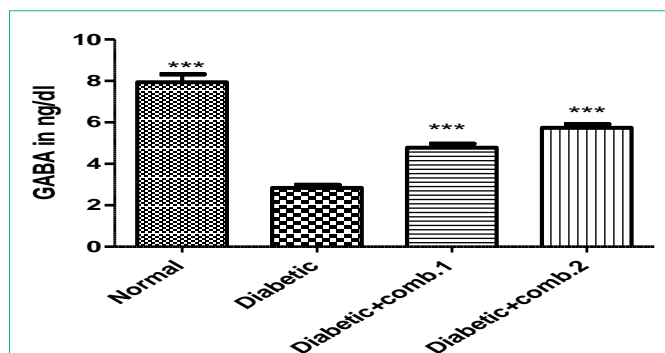


Figure 8: Effect of treatment of combination of drugs for eight weeks on brain GABA level in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

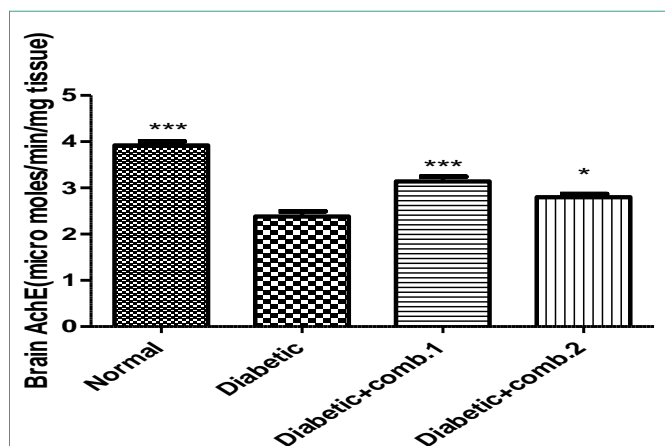


Figure 9: Effect of treatment of combination of drugs for eight weeks on brain AChE level in diabetic rats. Values are represented as mean \pm SEM (n=5). One Way ANOVA followed by Tukey's Multiple Comparison Test. ***P<0.001, P<0.05 Vs diabetic control group. Comb.1, gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine; Comb.2, gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine.

Estimation of gamma amino butyric acid (GABA)

The GABA level in brain of normal and diabetic rats was found to be 7.94 ± 0.37 ng/dl and 2.84 ± 0.143 ng/dl respectively and the GABA level of diabetic rats was significantly reduced ($P < 0.001$) as compared to normal control rats. GABA level in diabetic rats treated with comb.1 and comb.2 was found to be 4.78 ± 0.19 ng/dl and 5.74 ± 0.172 ng/dl and same was significantly increased when compared to diabetes control rats (Figure 8).

Estimation of acetylcholinesterase (AChE)

The AChE level in brain of normal and diabetic rats was found to be 3.920 ± 0.086 μ moles/min/mg and 2.38 ± 0.1 μ moles/min/mg respectively and the AChE level of diabetic rats was significantly reduced ($P < 0.001$) as compared to normal control rats. AChE level in diabetic rats treated with comb.1 and comb.2 was found to be 3.14 ± 0.103 μ moles/min/mg and 2.8 ± 0.070 μ moles/min/mg and same was significantly increased when compared to diabetes control rats (Figure 9).

Discussion

The current study has used comb.1 containing gamma linolenic acid, alpha lipoic acid, phloroglucinol, benfotiamine and Comb.2 containing gamma linolenic acid, alpha lipoic acid, allantoin, benfotiamine to alleviate the persistence of cognitive impairment in diabetic rats.

The metabolised product of gamma linolenic acid undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids (prostaglandins of series 1 and leukotrienes of series 3). Gamma linolenic acid and its metabolites also affect expression of various genes where by regulating the levels of gene products including matrix proteins. These gene products play a significant role in immune functions and also in cell death [20]. Lipoic acid is a cofactor essential in mitochondrial metabolism with anti-oxidant and anti-inflammatory activity. The mechanisms of action of alpha lipoic acid in experimental diabetic rats include reduction of oxidative stress along with improvement in nerve blood flow, nerve conduction velocity and several other measures of nerve functions [21]. Benfotiamine has been shown to prevent the progression of diabetic complications probably by increasing tissue levels of thiamine diphosphate and so enhancing transketolase activity and it is also found to inhibit Advanced Glycation Endproducts [22]. It was shown that benfotiamine (S-benzoylthiamine O-mono phosphate) possesses anti-inflammatory effects [23]. Phloroglucinol has been reported to have inhibitory activity in the formation of Advanced Glycation Endproducts (AGEs) and also provides anti-hyperglycemia and good anti-oxidant activity [24,25]. Allantoin shows anti-diabetic effects by modulating antioxidant activities, lipid profile and by promoting release of Glucagon Like Peptide (GLP-1), thereby improving the function of β - cells maintaining normal insulin and glucose level. Allantoin has also been found to increase Nitric Oxide (NO) level [26].

We have observed a significant reduction in the body weight of the diabetic rats as compared to the normal control group. Oxidative degradation of amino acids causes loss of tissue proteins and lipolysis which can cause reduction in body weight in diabetes. Improvement in the weight loss may be due to increase in the rate of metabolism in cell by combination treatment and decrease in the oxidative stress causing preservation of tissue protein by decreasing the muscle wasting contributing to the recovery of body weight.

In the present study diabetic control rats showed a significant reduction in falling latency on rota rod apparatus. The severity of diabetic neuropathy has been associated with decreased muscle strength in both type 1 and type 2 diabetes and weak grip strength in grip strength meter indicating poor motor/muscle activity [27]. Recently researcher have elucidate the influence of glial activation leading to cellular degradation and glutamate toxicity on altered behavioral activity in STZ induced diabetic rats. Time taken by diabetic rats to enter escape cage of Barne maze apparatus was significantly higher when compared with normal and treated rats. The decline in spatial navigational learning and memory in diabetes rats can be due to hyperglycemia which causes distortion in neurotransmitters level. In Type 1 diabetes mellitus an elevation in the glucose level is directly proportional to the rate of glycosylation of hemoglobin causing the formation of GHb (glycosylated hemoglobin) over previous four

weeks to three months. Marked increase in percentage of GHb has been reported in previous studies in diabetic rats [28]. GHb has been implicated in various diabetic microvascular complications like cognitive impairment, neuropathy, nephropathy, retinopathy etc. Glycosylation may lead to the formation of oxygen-derived free radicals in diabetes mellitus and its level can be considered as one of the important marker of oxidative stress. Therefore, in the present study we have measured the level of GHb as a marker of diabetes induced oxidative stress. Our results showed that the level of GHb in diabetic rats was significantly higher as compared to the normal rats. Treatment with the combination drugs significantly reduced HbA1c suggesting an improvement in glycaemic control.

Diabetes mellitus causes distortion in neurotransmitters level in brain which are responsible for learning, memory and many other activities. Dysfunction of neurotransmitters can be due to hyperglycemia, various vascular diseases and insulin resistance. In current study we found that the level of dopamine and serotonin in diabetic rats were significantly increased when compared to normal control rats whereas the level of nor-adrenaline, GABA and AchE were significantly decreased in diabetic rats when compared to normal rats. Treatment with combination restored the altered transmitter levels.

Conclusion

The combination contains drug acting by multiple mechanisms like gamma linolenic acid, alpha lipoic acid and benfotiamine which have been used since long. Addition of allantoin or phloroglucinol drug with good safety profile can be a safe for long term administration in diabetics to successfully delay the developments of cognitive impairment.

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